MINIREVIEW

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Human Enteroids/Colonoids and Intestinal Organoids Functionally Recapitulate Normal Intestinal Physiology and Pathophysiology*

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Identification of Lgr5 as the intestinal stem cell marker as well as the growth factors necessary to replicate adult intestinal stem cell division has led to the establishment of the methods to generate "indefinite" *ex vivo* primary intestinal epithelial cultures, termed "mini-intestines." Primary cultures developed from isolated intestinal crypts or stem cells (termed enteroids/colonoids) and from inducible pluripotent stem cells (termed intestinal organoids) are being applied to study human intestinal physiology and pathophysiology with great expectations for translational applications, including regenerative medicine. Here we discuss the physiologic properties of these cultures, their current use in understanding diarrhea-causing host-pathogen interactions, and potential future applications.

The absence of drugs to treat many human diseases demonstrates the limits of clinically relevant data obtained from studies performed in animal models and cancer cell lines, which has contributed to the failure of ~90% of all potential drug therapies in human clinical trials (1). Development of engineered human tissues and organ systems derived from stem cells is providing new model systems to facilitate drug development. These systems have been pursued with the assumption that normal human tissues differ in terms of response, efficacy, and toxicity when compared with animal or cancer-derived cell models. In the intestine, stem cells are present at the base of the intestinal crypts (Fig. 1). The lifespan of enterocytes, from the time of their appearance at the base of crypt and migration along the crypt-villus axis to the time of shedding from the villus tip (or colonic surface), is relatively short (~5–7 days). Thus, constant division of intestinal stem cells is required to maintain the intestinal epithelium. The molecular identity of intestinal stem cells was debated until 2007 when Hans Clevers and colleagues (2) discovered a population of fast cycling intestinal stem cells that uniquely express high levels of Lgr5 (mouse leucine-rich repeat-containing G protein-coupled receptor 5). The identification of $Lgr5^+$ stem cells and discovery of the growth factors required for stem cell homeostasis made it possible to establish *ex vivo* adult intestinal epithelial cultures or "mini-intestines," which can be passaged long-term without significant genetic or physiologic changes (3, 4).

Human Intestinal Stem Cell-derived Mini-intestines: Enteroids *Versus* Organoids

Currently, the two primary ways to generate human mini-intestines include (*a*) isolation of intestinal crypts, which contain human adult stem cells, from donors or (*b*) use of human embryonic or inducible pluripotent stem cells (iPSCs).[3] Both methods have been described in detail and reviewed elsewhere (3–5). Intestinal crypts, containing human adult stem cells, can be isolated either from surgically resected tissue or from biopsies, embedded in Matrigel, and cultured as *ex vivo* self-perpetuating three-dimensional primary cultures in growth factor-enriched media. The critical growth factors for long-term intestinal culture include Wnt3a, R-spondin, and Noggin (4, 6, 7). Although much of our understanding of the role of Lgr5 has come from mouse studies, successful culture of human enteroids requires R-spondin, suggesting that $LGR5^+$ (human) cells must be present. Initially, these cultures produce polarized three-dimensional spheroid-like structures with the apical domain facing inside newly formed lumens and basolateral surfaces in contact with the Matrigel and external media (Fig. 1). These are referred to as enteroids (derived from small intestine) or colonoids (derived from colon) and contain only epithelial cells types derived from the crypt-based stem cells (8). The major epithelial cell types present in small intestinal enteroids, in addition to $LGR5^+$ stem cells, include Paneth cells, enteroendocrine cells, enterocytes, and goblet cells in relatively the same proportions as are present in the intestine itself (7). Enteroids vary in size (several μm to mm) and usually double in size over 24 h. After several (~5) days in culture following withdrawal of the critical growth factors, enteroids differentiate to form a villus-like epithelium composed of mature enterocytes, enteroendocrine cells, and goblet cells, whereas $LGR5^+$ stem cells and transit-amplifying zone cells are lost. Crypt-derived enteroids represent the fastest approach to generate mini-intestines.

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[3] The abbreviations used are: iPSC, inducible pluripotent stem cell; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; NHE, sodium-hydrogen exchanger; RV, rotaviral; CBC, crypt base columnar; AMCA, N_ϵ-7-amino-4-methylcoumarin-3-acetic acid.

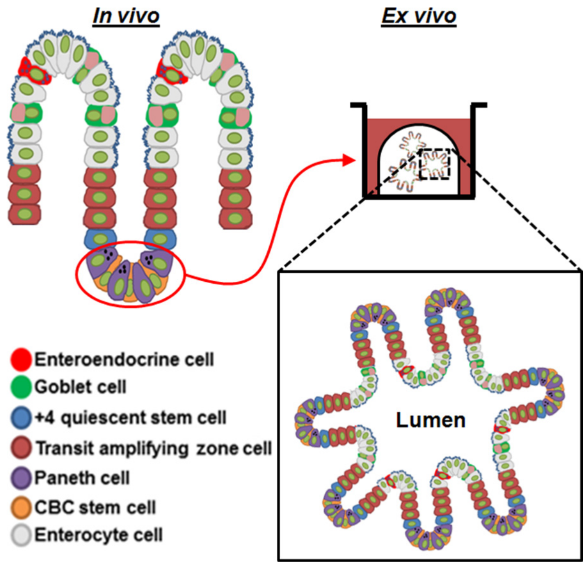

FIGURE 1. **Intestinal stem cells (*i.e.* CBC) located at the base of the crypts can be cultured *ex vivo* to generate indefinitely propagating enteroid cultures.** The enteroid epithelium is composed of the same intestinal cell types that exist *in vivo*.

Human three-dimensional intestinal tissue cultures, termed organoids, can also be generated *in vitro* from iPSCs. Based on the methodologies developed by Spence *et al.* (5), intestinal organoids are generated from iPSCs that are differentiated to form mid- and hindgut tissue. From the mid- or hindgut epithelial cell layers, three-dimensional spheroid buds are further cultured in Matrigel along with pro-intestinal growth factors. These spheroids contain intestinal cells, including mesenchymal and all major intestinal epithelial cell types, but lack enteric nerves and immune cells. In contrast to enteroids, LGR5 is not robustly expressed during early stages of organoid "development," but is expressed in discreet epithelial domains after prolonged culture (5). The similarities and differences between enteroids and organoids have been reviewed by us and others (9–11).

Potential applications for these *ex vivo* intestinal models include further understanding of normal digestive physiology, developmental biology, and pathophysiology of intestinal diseases including inflammatory bowel diseases, cystic fibrosis (CF), host-pathogen interactions, and regenerative medicine (9, 12–16). Mini-intestines are also being used to study drug absorption/toxicity, intestinal wound healing, genetic engineering, and transplantation. To be suitable for these applications, enteroids and organoids must accurately reproduce major aspects of normal intestinal epithelial physiology and reflect key features of the pathophysiology of human intestinal disorders. Assays to measure these functions are being established with several commonly used biochemical assays. For measuring drug toxicity, our laboratories showed that indomethacin, a non-steroidal anti-inflammatory drug that has intestinal side effects including vomiting, diarrhea, and constipation, causes cell death in human small intestinal enteroids (data not shown). However, the mechanism responsible for this effect remains to be determined. These data suggest that human enteroids recapitulate intestinal drug toxicity in humans.

Differentiation

Differentiation refers to the ontogeny of enterocyte and secretory cell lineages from crypt-based stem cells along the vertical crypt/villus axis (4). In the movement along the crypt/villus axis, epithelial cells differentiate into enterocytes, enteroendocrine cells, and goblet cells, as well as exhibiting structural changes with formation of a villus structure (surface region in the colon). Enterocytes along the crypt/villus axis exhibit distinct differentiation states between enterocytes residing in the transit-amplifying zone (less differentiated) when compared with those in the villi (or surface in colon) that are more differentiated. There are several common features of cell behavior in the different intestinal segments (*i.e.* duodenum, jejunum, ileum, colon), each of which exhibits structural and functional diversity. Although the lifespan of enterocytes and colonocytes is about 5–7 days, the lifetime of Paneth cells, which sandwich the stem cells at the crypt base to form a niche for the stem cells, is much longer (>30 days). Both enteroids and organoids reproduce the same lifespan for all cell types in culture as occurs in intact tissue (5, 7).

The gradient of WNT3a and EGF signaling is highest at the base of the crypt, where they secure stem cell survival and proliferation into the transit-amplifying zone, which contains mainly undifferentiated, proliferating enterocytes or colonocytes. There is a gradual decrease in Wnt3a and EGF signaling from the crypt base toward the gut lumen, which is accompanied by increased signaling gradient of bone morphogenic protein, BMP (responsible for villus enterocyte differentiation), and Notch, which differentiates epithelial cells toward secretory phenotypes, including mucus-producing goblet cells, hormone-producing enteroendocrine cells, Tuft cells, M cells (which require RANkL (receptor activator of NF-κB ligand) as an additional growth factor), and L cells (6, 17, 18).

Because human enteroids do not produce significant amounts of WNT3a, EGF, or several other growth factors essential for stem cell division and cell proliferation, terminal differentiation is achievable in enteroids by withdrawal of WNT3a for 5 days (6). This leads to loss of LGR5, a decreased cell proliferation, and the appearance of secretory and absorptive cell lineages, including goblet and enteroendocrine cells (7). Differentiation also transforms immature crypt-like enterocytes into differentiated nutrient-absorptive cells that produce digestive enzymes, including dipeptidyl peptidase IV (DPPIV), sucrase-isomaltase (SI), lactase, and alkaline phosphatase,[4] similarly to villus cells in human adult small intestine.

In contrast, iPSC-derived organoids are more similar to fetal human intestine than adult intestine based on limited gene profiling that compared enteroids generated from human fetal and adult small intestine (19). A recent study reported that adult-like intestinal organoids could be derived from iPSCs; however, these organoids were isolated from iPSCs grown as teratomas to promote differentiation (20). Importantly, the different methods used to generate organoids and maintain their growth *in vitro* confound comparisons from one study to another. In

[4] N. C. Zachos, O. Kovbasnjuk, J. Foulke-Abel, J. Yin, J. In, S. E. Blutt, H. R. de Jonge, M. K. Estes, and M. Donowitz, unpublished results.

general, the generation of functional, mature cells or tissues from iPSC cultures has proven challenging across many tissue types (21–24). The difficulty in achieving significant differentiation in such preparations is linked to their inability to recapitulate normal function of many PSC-derived cells and tissues. For example, iPSC-derived pancreatic endocrine cells are unable to secrete insulin in response to glucose (25), and liver hepatocytes have an enzyme expression profile that is fetal in nature (26). Because the mesenchymal cells present in organoids constantly produce growth factors that support cell proliferation but not differentiation, the inability to remove growth factors or mimic the growth factor gradient present in the crypt-villus axis necessary for differentiation results in the less mature status of the organoids.

Until now, characterization of differentiation in enteroids and organoids has largely been restricted to the expression of proteins that are differentially expressed in the villus/surface and crypt and longitudinal segmental differentiation. Formation of villi, a major characteristic of the differentiated intestine, has not been achieved with enteroids and only achieved with organoids by implanting the organoid beneath the kidney capsule of the mouse (27). These data suggest that the presence of mesenchyme and proper vasculature may be needed to activate the genes involved in villus formation; however, future studies are needed to understand how a crypt/villus axis develops under these conditions. Other data suggest that mechanical stress has been suggested to be the important factor for villus formation. For example, Caco-2 cells grown on Transwell filters under static conditions form a confluent monolayer that becomes terminally differentiated ~3 weeks after confluency. In contrast, recreation of a gut microenvironment by flowing fluid, which produces low shear stress, and exerting cyclic strain that partially mimics peristalsis stimulates the development of Caco-2 columnar epithelium that rapidly polarizes and spontaneously grows into folds that resemble the structure of intestinal villi and produces functional tight junctions that serve as a barrier to small molecules (28). Because luminal perfusion alone does not stimulate villus formation in Caco-2 monolayers, cyclic strain appears to be a necessary condition. In contrast, a more recent study demonstrated that development of muscle layers around the embryonic gut is necessary for villus formation (29). Thus, constraint provided by the muscle tube, rather than spontaneous contractility of smooth muscle, may be the factor required for epithelial folding (29). Because both three-dimensional enteroid and organoid cultures spontaneously form morphologically well defined crypts, further studies are necessary to dissect the molecular mechanisms (*e.g.* mesenchyme, vasculature, cyclic strain, contraction) that regulate "villification" of enteroids, which probably represent a further stage of differentiation.

Mini-intestine Segment-specific Patterning

Division of the gastrointestinal tract into multiple distinct segments, each of which exhibits structural and functional diversity, raised the question of whether such patterns are retained in *ex vivo* mini-intestine cultures. In fact, a recent study demonstrated that small intestinal enteroids differentially express proteins that are specific to the intestinal segment (*i.e.* duodenum, jejunum, ileum) from which they are derived (30). For example, bile acids are taken up exclusively in the distal ileum prior to release in the portal venous circulation for return to the liver. This occurs in distal ileal enterocytes through entry via the apical Na^+-dependent bile acid transporter (ASBT, SLC10A2) and secretion from the enterocytes to the portal blood by the basolateral transporter, organic solute transporter β subunit (OSTB). Expression of both ASBT and OSTB is found only in differentiated enteroids derived from the ileum (30). In contrast, ileal enteroids lack expression of the transcription factor GATA4 in both the differentiated and undifferentiated state. As in native tissue, GATA4 expression in the proximal small intestine correlates with the suppression of ileal predominant proteins and controls regional epithelial cell identity in the adult intestinal epithelium in a differentiation-independent manner. These data support the current view that protein expression profiles are intrinsically imprinted within the human intestinal stem cells (hISCs) of each intestinal segment that is maintained *ex vivo* in long-term enteroid cultures. Although crypt-derived human enteroids express segment-specific genes, co-expression of GATA4/GATA6 and Paneth cells in iPSC-derived organoids suggests that they are most similar to the proximal small intestine. However, through manipulation of transcription factors, Wells and co-workers have produced both duodenal and colonic like mini-intestines from iPSCs.[5]

Significant differences exist in the segment-specific gene expression profile between human and mouse intestine and corresponding enteroid cultures (30). This discovery is important as it may partially explain the lack of consistent results of preclinical mice studies to outcomes in humans. For instance, the gene encoding the basolateral iron absorptive membrane protein ferroportin (SLC40A1) is detected exclusively in mouse duodenum and corresponding enteroids, whereas this protein is expressed in both the duodenum and the ileum in human intestine (30). Additionally, lactase is more highly expressed in the mouse jejunum and in murine jejunal enteroids when compared with very low expression in mouse duodenum and ileum and in the corresponding enteroids. In contrast, lactase is expressed in both human duodenal and ileal enteroids, which supports data on lactase immunostaining in human tissue from corresponding intestinal segments (30). These contrasting data further support that mouse models may have limitations in their ability to accurately model human intestinal physiology. Moreover, these contrasting data between the mouse and human suggest that further characterization of the molecular profile of normal human intestinal tissue, in parallel with human enteroids or organoids, will provide valuable insights via experiments designed to study normal human physiology and intestinal diseases, and for the development of novel therapeutic compounds.

Transport Function

The two major functions of enterocytes are digestion/absorption of nutrients and water/electrolyte homeostasis. The transport of electrolytes, which becomes abnormal in diarrheal

[5] J. M. Wells and co-workers, personal communication.

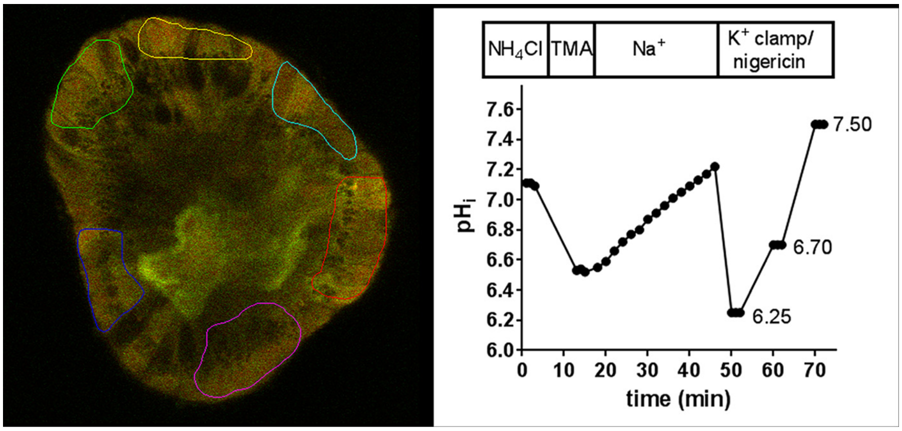

FIGURE 2. **Human enteroids as a model to study NHE3 activity.** Human duodenal enteroid was loaded with the pH-sensitive dye, SNARF-4F (*left*; pseudocolor image), and imaged using a two-photo microscope (Olympus). NHE3 activity, which is inhibited by ethylisopropyl amiloride or S3226, is defined as Na^+-dependent intracellular alkalinization following an acid load in the presence of 50 μM HOE-694 (inhibits all other plasma membrane NHE isoforms) (61). To measure NHE3 activity (*right panel*), enteroids were acid-loaded by pulsing NH_4Cl followed by Na^+-free, tetramethylammonium (*TMA*) solution, resulting in intracellular acidification. After the addition of Na^+-containing solution, brush-border NHE3 exchanged extracellular Na^+ for intracellular H^+. Intracellular pH of enteroids was calibrated after final exposure to K^+ clamp solution containing nigericin at various pH values (*e.g.* 6.25, 6.70, 7.50). Measurements were calculated from regions of interest (*multi-colored traces* in *left pseudocolor image*) using MetaMorph Image Analysis software.

diseases, includes luminal Cl^- and HCO_3^- secretion and Na^+ and Cl^- absorption. The former occurs in cells expressing apical cystic fibrosis transmembrane conductance regulator (CFTR), basolateral NKCC1 (provides Cl^- for secretion), and K^+ channels (for intracellular charge maintenance). NaCl absorption occurs in cells with brush-border sodium-hydrogen exchanger, NHE3, which is functionally linked to the brush-border Cl^-/HCO_3^- exchangers, DRA (SLC26A3) (31) and, to a lesser extent, PAT-1 (SLC26A6).

Using two-photon microscopy with a pH-sensitive dye to measure intracellular pH (Fig. 2) and a luminal dilatation assay (12), physiological regulation of intestinal Na^+ absorption and Cl^- secretion has been quantitated in human small intestinal enteroids (13). As shown in Fig. 2, NHE3 activity is measured as the rate of exchange of extracellular Na^+ with intracellular H^+. Under basal conditions, there is apical NHE3 activity but little fluid secretion (32). Elevation of intracellular cAMP (by forskolin treatment) activates apical Cl^- secretion by CFTR and significantly increases the luminal fluid secretion, which can be measured by luminal dilatation (12). This process is accompanied by inhibition of NHE3 activity (32). Fluid secretion can be prevented by inhibiting CFTR with specific inhibitors $CFTR_{inh}$-172 and GlyH (33, 34). Thus, human enteroids carry out Na^+ absorption and Cl^- secretion, which appears to be regulated as occurs in normal digestive physiology (*e.g.* meal-related secretin release). Another example of transport function in enteroids that mimics what occurs in intact intestine is uptake of peptides demonstrated with the fluorescently labeled dipeptide, D-Ala-Lys-AMCA (5).

Another important lesson learned from enteroid cultures is derived from the study of CF patients and relates to genetic and biologic diversity. Deletion of Phe-508 (ΔF508) is the most prevalent CFTR mutation and is present in ~90% of CF patients. This mutation causes protein misfolding, endoplasmic reticulum retention-associated degradation of CFTR, and reduced trafficking and stability that result in marginal apical membrane expression of a partially functional Cl^- channel. Therapy based on combinations of pharmacological correctors and potentiators has raised hope for better medical outcomes in CF patients. This combined therapy enhances the delivery of functional ΔF508-CFTR to the apical membrane and improves ΔF508-CFTR-dependent swelling of enteroids upon forskolin treatment to 30–60% of that observed in enteroids with wild type CFTR (12). In enteroids expressing another nonfunctional, truncated CFTR variant, Cl^- channel activity is not rescued by this drug regimen. These data strongly advocate for the development of personalized approaches to treat human disease and support the importance of enteroid models as a therapy-testing platform for individual patients to effectively treat their intestinal disorders (35). In addition, these studies support the need to create an atlas of transport protein expression and localization in normal human intestinal tissue, which should be designed to capture genetic and phenotypic biodiversity.

Host-Pathogen Interactions

Enteroids are showing great promise as models to study the interaction between intestinal pathogens and the intestinal epithelium. To date, human enteroids have been shown to model human RV infection, cholera toxin effect on transport, and several aspects of enterohemorrhagic *Escherichia coli*-related human diarrhea (9). RV replicates and produces infectious virus in human enteroids and organoids, with viral replication increasing over 96 h (13, 36). Rotavirus infection causes very rapid inhibition of NHE3, which might be a significant contributor to RV-induced diarrhea, the pathophysiology of which remains poorly understood (37). Cholera toxin exposure for several hours duplicates the inhibition of NHE3 and stimulation of fluid secretion that occurs over a shorter time frame with forskolin (9). Exposure of enteroids to the enterohemorrhagic *E. coli* serine protease, EspP, which possesses cytotoxic activity, significantly alters the distribution of F-actin in both apical and basolateral membranes of enterocytes with significant actin remodeling in microvilli and the terminal web as well as significant widening of the lateral intracellular space by destroying the cell-cell contacts along the basolateral membrane below the tight junctions (38). Recently, intestinal organoids have been used by two laboratories to study *Clostridium difficile* infection. The Spence laboratory demonstrated that luminal exposure of *C. difficile* in intestinal organoids results in increased paracellular permeability with the *C. difficile* toxin TcdA having a more potent effect than TcdB (39). In addition, the Worrell laboratory found that luminal *C. difficile* infection reduces NHE3 and MUC2 protein levels, which may partially explain how *C. difficile* creates a luminal environment to enhance colonization (40, 41). Further studies of infections of human enteroids are expected to lead to identification of novel mechanisms of human response to infection with a wide spectrum of human commensals as well as bacterial, viral, and parasitic pathogens, many of which currently lack animal models. New models of chronic infection, including those that may lead to understanding intestinal development and tumor biology, are also possible, with evidence already being reported for a bacterial infection model to study epithelial-mesenchymal transition or tumorigenesis (42).

Model Development: Variations in Culture Methods

Recently, several modifications from the original protocol established by Sato *et al.* (4) have resulted in the use of growth factors from different sources to simplify the process of generating mouse and possibly human enteroids (43, 44). Although these changes may have some advantages, particularly for specific intestinal segments, a concern is that modifications may introduce significant variability in gene expression from passage to passage and from one laboratory to another. Similar problems have been documented for the Caco-2 intestinal epithelial cell culture model, in which the quantitative aspects of expression of transporter profiles have become widely divergent (45). This has made it difficult to validate functional assays with these cells including use for testing of intestinal drug efficacy and toxicity. We propose that protocols for growth, differentiation, and conditioned media should be standardized so that differences in gene expression between different enteroid cultures reflect the true biologic diversity of the human population rather than differences in cell culture medium/conditions.

Characterization of Crypt-based Stem Cell Subtypes

Currently, one of most controversial areas in intestinal stem cell biology is the delineation and characterization of multiple intestinal stem cell populations. It is well established in mouse models that the fast cycling crypt base columnar (CBC) intestinal stem cells express Lgr5 (mouse). There have been numerous attempts to prove the existence of so-called "quiescent" or "reserve" stem cells that divide infrequently and mainly in response to gut injury (*e.g.* radiation). The current state of the field has been recently reviewed in detail (46). The authors note that numerous lineage-tracing experiments indicate that CBC cells behave as interconvertible multipotent intestinal stem cells (2, 47–50). However, regardless of the type of injury (*e.g.* radiation), the stem cells that repopulate the intestinal epithelium appear to be Lgr5$^+$. Studies in human enteroids are needed to determine whether these new models of intestinal development will confirm results in mice or whether new mechanisms of intestinal homeostasis will be discovered.

Intestinal Organogenesis

Studies using enteroids and organoids are advancing our understanding of the role of the epithelium in human intestinal physiology and pathophysiology. Mouse and human enteroids are only composed of epithelial cell types and are thus useful to understand intestinal epithelial cell function in the absence of other cell types. For example, studies in mouse enteroids have confirmed a cell-autonomous role of CFTR in the regulation of intracellular pH, and a sustained alkaline pH_i in CF may underlie the abnormalities in goblet cell and Paneth cell degranulation reproduced at the enteroid level (51). The mesenchymal cells that accompany intestinal organoids provide essential growth factors, and as three-dimensional cultures, organoids are composed of an epithelial monolayer juxtaposed to the surrounding mesenchyme. Furthermore, after being grown under the mouse kidney capsule, the mesenchymal cells differentiate into smooth muscle and sub-epithelial myofibroblasts, the latter providing some of the extracellular matrix for the epithelium (52). Due to the cellular diversity in intestinal organoid models, organoids grown under the kidney capsule exhibit structural characteristics similar to those that exist in the mature *in vivo* intestine, including villi with lamina propria and well organized crypts (27). Whether this architecture replicates *in vivo* functions remains to be established. Current models of intestinal enteroids and organoid mini-intestines lack enteric nerves and immune cells, and one goal is to add these components to develop a more "complex" *in vitro* intestinal model using co-culture approaches. An example of this is supported in a study by Lahar *et al.* (52), which demonstrated that long-term culture of the human small intestinal epithelium required the presence of sub-epithelial myofibroblasts, even when exposed to Wnt3a-containing media. More recently, the Grikscheit laboratory, expanding upon the techniques developed by Evans *et al.* (53) and Vacanti *et al.* (54) in the rat, demonstrated successful regeneration of the human small intestine (termed tissue-engineered small intestine or TESI) from collagenase-digested "organoid units" that contain LGR5$^+$ stem cells and mesenchyme (55). Tissue-engineered small intestine exhibits a villus/crypt axis, but unlike intestinal organoids, it also contains enteric neurons, perhaps paving the way for neuronal cell replacement therapy in Hirschsprung disease (56). In addition, intact organoids injected into the colon of dextran sulfate sodium-treated mice resulted in organoid implantation and repair of the damaged epithelium (19). The evolution of the enteroid/organoid models to include sub-epithelial myofibroblasts, smooth muscle cells, enteric nerves, immune cells, microbiome, and endothelial cells is expected to help facilitate our understanding of complex intestinal organ development. In addition, methods have been established to alter gene expression either by overexpression or shRNA-mediated knockdown using lipophilic transfection reagents and virus-mediated transduction (14, 57). These new models may provide valuable insight into developing methodologies for intestinal regeneration that lead to therapeutic options including repair of the damaged epithelium that occurs in ulcerative colitis, microvillus inclusion disease, or intestinal transplantation to treat short bowel syndrome (15, 58).

Future Needs

In conclusion, development of the methodology necessary to culture human intestinal enteroids and organoids has led to advances in the understanding of normal human intestinal stem cell physiology. Although each model is composed of a differentiated epithelium and can replicate the segment specificity of the small intestine, as pointed out by Brugmann and Wells (59), organoids can be developed into a more complex organ system due to differentiation of their supporting mesenchymal cells and thus may be useful in developmental studies or for regenerative medicine. In contrast, human enteroids can be generated from all segments of the small intestine and colon and maintain the molecular and physiological profiles of each segment. Transport studies have shown that enteroids are functionally regulated under pathophysiological conditions and can provide insight into mechanisms of disease. Despite the great potential that exists for each model system, the field remains quite new and is rapidly progressing. Modifications to established protocols are likely to uncover new conditions required for specific aspects of division and differentiation of these preparations and reveal new translational applications. A recent example is the demonstration that inhibition of FOXO1 in human gut organoid cultures yields functional insulin-producing cells (60). Detailed documentation and comparisons will be necessary to properly define the genetic, biochemical, and physiologic aspects of the resulting enteroids and organoids. These findings will be necessary as these models are used to answer questions about human intestinal physiology and pathophysiology and for drug development and regenerative medicine, among other applications.

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